Ready-to-use 3D Human Cortical Spheroids

SP3D-HCS
Cat. #SP3D-1520

Product Description

Damage to the central nervous system (CNS) has a life-threatening impact on humans, and there is a high demand for relevant models to study CNS diseases and therapeutic strategies. Mature neurons and astrocytes in the brain together form a complex three-dimensional network system [1]. Astrocytes *in vivo* regulate blood flow, provide energy to neurons, and supply the building blocks of neurotransmitters, which support the formation of functional synapses [2]. The complex cellular arrangement of the central nervous system are unlikely to be recapitulated in 2D cultures where cells display the planar morphology and have cellular interactions only in the lateral direction. To create a more biologically relevant *in vitro* brain model, ScienCell has developed ready-to-use 3D human cortical spheroids (SP3D-HCS) comprised of primary human neurons and astrocytes. SP3D-HCS are generated by coculturing neurons and astrocytes at a 1:7 ratio in 3D culture. Immunofluorescence analysis revealed that the 3D human cortical spheroids maintain direct cell-cell interactions between neurons and astrocytes and form functional synapses throughout the spheroids (Figures 1 and 2). Importantly, in the defined, serum-free spheroid medium, the neurons are networked with quiescent astrocytes, a phenotype closely resembling *in vivo* astrocytes. The quiescent astrocytes are critical for synapse function and neural development. This advanced and ready-to-use cortical spheroid is highly suitable for the study of CNS functions, diseases, and therapeutics.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-1520	1	Human Cortical Spheroids (SP-HCS)	1 x 10^6 cells	Liquid nitrogen
3D-1521	1	3D-Neuronal Spheroid Medium – basal (3D-NSpM)	200 mL	2-8 °C
3D-1562	1	3D-Neuronal Spheroid Supplement (3D-NSpS)	4 mL	-20 °C
0583	1	Penicillin/Streptomycin Solution (P/S)	2 mL	-20 °C
0343 (or) 0353 (or) 0383	1	Ultra-Low Binding Culture Plates (24-, 48-, or 96- well plate)	1 plate	RT

Quality Control

SP3D-HCS is tested for the formation of functional and uniform 3D human cortical spheroids according to the included protocol. All components are negative for bacterial and fungal contamination.

Product Use

SP3D-HCS is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping
SP-1520, 3D-1562, 0583 are shipped on dry ice. 3D-1521, and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Zhuang P, Sun AX, An J, Chua CK, Chew SY. (2018) "3D Neural Tissue Models: From Spheroids to Bioprinting." *Biomaterials.* 154: 113-133.
[2] Eroglu C, Barres BA. (2010) "Regulation of synaptic connectivity by glia." *Nature.* 468(7321): 223-231.

Procedure:
Step I: Preparing the complete 3D culture medium

1. Thaw 3D-neuronal spheroid supplement (3D-NSpS; Cat. #3D-1562), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix 3D-NSpS, and P/S solution into the 3D-neuronal spheroid medium (3D-NSpM medium; Cat. #3D-1521) by gently swirling the medium bottle around.

    a. 3D-NSpM medium is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-NSpM medium to **room temperature** before use.
    c. When stored in the dark at 4°C, the complete medium is stable for one month.

Step II: Thawing and maintaining the ready-to-use 3D spheroids

2. One frozen vial contains $\geq 1 \times 10^4$ spheroids, which is sufficient for plating into half of a multi-well plate (e.g. 24-, 48-, and 96-well ultra-low binding culture plate).

3. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

4. Carefully remove the cap without touching the interior threads. Gently pipette spheroid suspension up and down for **two times** to disperse potential spheroid aggregates.

5. Gently transfer the spheroid suspension into a fresh 50 mL conical tube.

6. Add the 12 mL of 3D culture media to the above 50 mL conical tube.

7. Resuspend spheroids in 3D culture media by gently pipetting up and down for ~ 5-7 times using a serological pipette.

    Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid bubble formation.

8. Aliquot the suggested volumes (see **Table A, column 2**) of spheroid suspension into each well of the ultra-low binding plate (24-, 48- or 96-well plate).

Table A: An Example of Suggested Medium Volumes

1	2
Plate formats	**Volume per well**
24-well	~ 1000 µL
48-well	~ 500 µL
96-well	~ 250 µL

9. Incubate spheroids at 37°C in a 5 % CO_2 incubator.

10. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated.

11. Next day, change 60-70 % of the top layer of the medium using a pipette by hand to remove the residual DMSO. (Do not use a vacuum aspirator) After 1st medium change, change 60-70% of the top layer of the medium every 4-5 days.

    Note: Spheroids are situated at the bottom of the well due to the viscosity of the 3D culture medium. Thus, centrifugation of the plates is not necessary, and spheroid loss will not occur by changing 60-70 % of the top layer of the medium by pipetting.

12. Monitor the health of spheroids every day under the microscope. Cortical spheroids are recovered and ready for your experiment after 24-48 hours post thawing (see Figure 1).

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Fig. 1 – Ready-to-use human cerebral cortical spheroids at 24-72 hours after thawing (taken at 100x magnification).

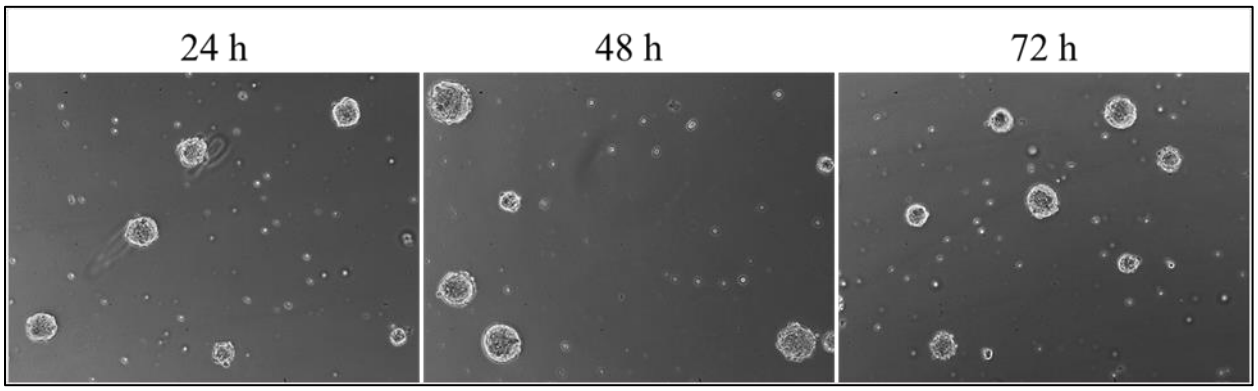

Fig. 2 – At days 7; immunostaining of the human cerebral cortical spheroids with the neuronal marker β-tubulin III, astrocyte marker GFAP, and synaptic marker SYN1 (taken at 400x magnification).